AN AUTORADIOGRAPHIC AND HISTOCHEMICAL INVESTIGATION OF THE GUT MUCOPOLYSACCHARIDES OF THE PURPLE SEA URCHIN (STRONGYLOCENTROTUS PURPURATUS)[1]

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The older literature dealing with regular echinoid anatomy and histology mentions gland cells in the gut wall (see Hyman, 1955). More recently, Stott (1955) reported that gland cells in the wall of the sea urchin gut secrete an acid mucus, while Fuji (1961) reported that some sea urchin gut glands produce an acid mucus and others produce a neutral mucus. In the present study, some newer techniques of autoradiography and histochemistry have been applied to all regions of the gut of the purple sea urchin, *Strongylocentrotus purpuratus*, to reveal the distribution and characteristics of the mucopolysaccharides occurring in the wall of the digestive tract.

At present, the terminology for sea urchin gut regions is not entirely settled. It is, therefore, necessary to define the terms used in this paper. In *S. purpuratus*, the small buccal cavity extends from the mouth opening in the center of the peristome to the approximate level of the nerve ring. Interradially, each of the five teeth perforates the wall of the buccal cavity. In each radius is an outpocketing of the buccal cavity wall (Figs. 1 and 2). There are five such structures alternating with and lying between the five teeth. These outpocketings have not been named before, and we shall call them the radial buccal diverticula. In each interradius, there is an outpocketing of the roof of the buccal cavity (Figs. 1 and 5). Although these five outpocketings have been called the lips of the pharynx (Delage and Hérouard, 1903; Stott, 1955), we shall call them interradial buccal diverticula. The pharynx begins at the approximate level of the nerve ring and extends to the aboral surface of the lantern. The esophagus, which has about the same diameter as the pharynx, begins at the aboral surface of the lantern and extends to its junction with the stomach. This junction is conspicuous, since the stomach diameter is several times the esophagus diameter. Near the junction of esophagus and stomach, a slender tube, the siphon, leaves the main course of the gut to run parallel to the stomach. The stomach and siphon together make a nearly complete circuit of the

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body to the beginning of the intestine. The siphon rejoins the main part of the gut near the junction of the stomach and intestine. The intestine doubles back on the stomach and makes a nearly complete circuit of the body aboral to the stomach. The intestine ends indistinctly with a short rectum which ascends to the aboral pole, and terminates at the anus.

Histologically, the entire digestive tract is composed of three layers. The inner lining is a tall columnar epithelium except in parts of the buccal cavity where it thins to a squamous epithelium. The layer covering the coelomic surface of the gut is a flagellated squamous epithelium, the visceral peritoneum. Between these two

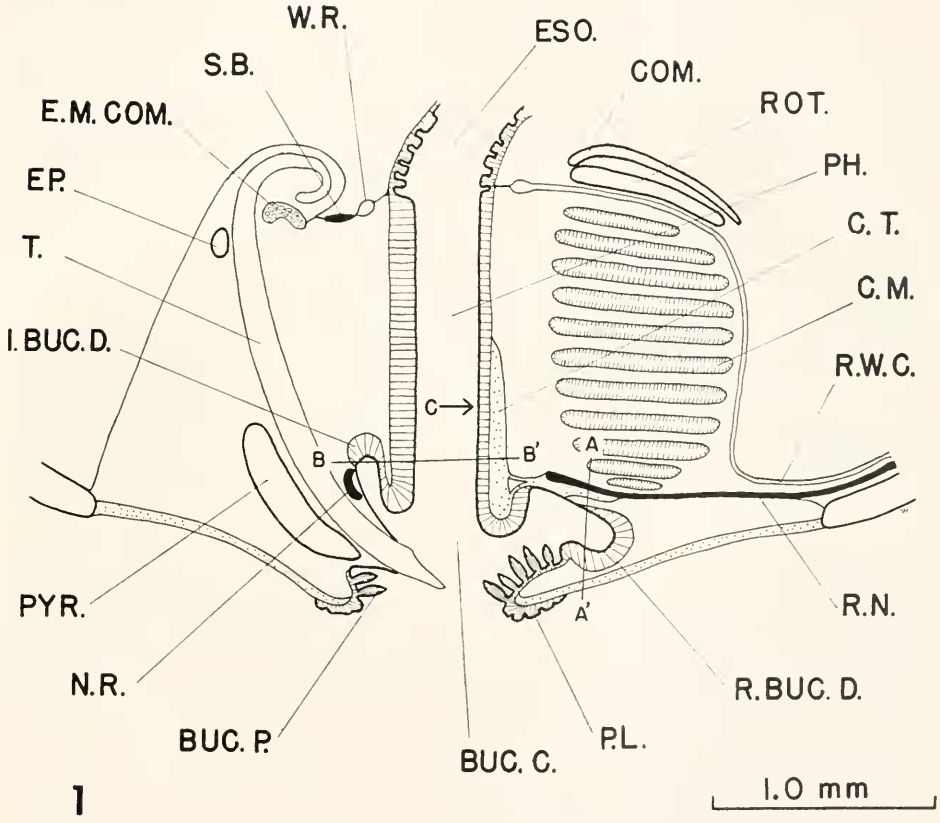

FIGURE 1. Vertical section through the Aristotle's lantern and adjacent structures of a 0.3-g. *S. purpuratus*. The right half of the figure is radial and the left half is interradial. The abbreviations, in clockwise order from the top of the figure, are as follows: ESO., esophagus; COM., compass; ROT., rotule; PH., pharynx, C. T., connective tissue; C. M., comminator muscle (these muscles are actually several times more numerous than depicted); R. W. C., radial water canal; R. N., radial nerve; R. BUC. D., radial buccal diverticulum; P. L., peristomial lip; BUC. C., buccal cavity; BUC. P., buccal papilla; N. R., nerve ring; PYR., pyramid; I. BUC. D., interradial buccal diverticulum; T., tooth; EP., epiphysis; E. M. COM., elevator muscle of compasses; S. B., spongy body; W. R., water ring. The haemal system is not shown in this figure.

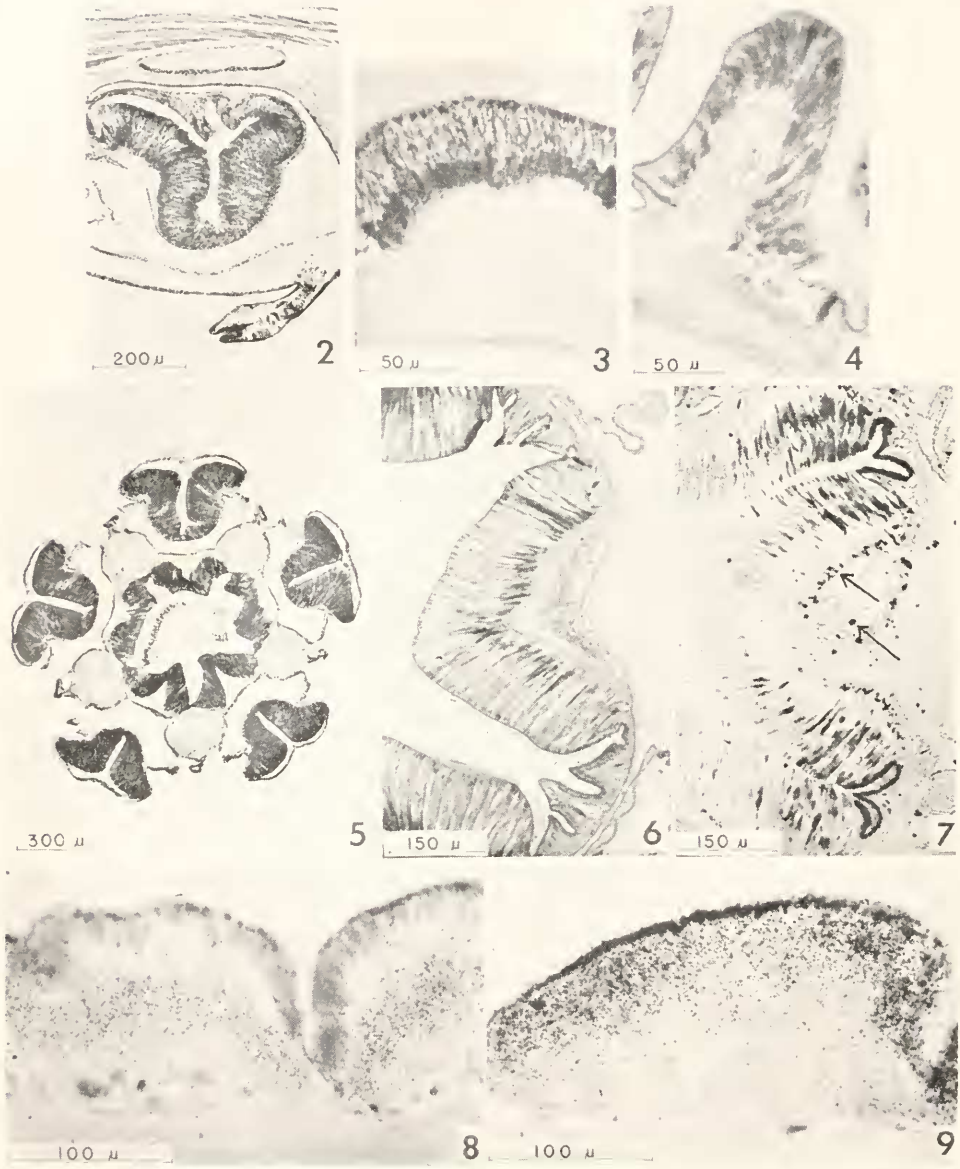

FIGURE 2. Cross-section of a radial buccal diverticulum and adjacent structures of a 0.3-g. urchin. The section is in the plane of A-A′ in Figure 1. From top to bottom are comminator muscles, radial nerve supported by connective tissue, radial buccal diverticulum surrounded by the peripharyngeal (or lantern) coelom, and peristomial membrane bearing a pedicellaria on its outer surface. Haematoxylin and eosin.

FIGURE 3. Vertical section of the peristomial lip of a 0.3-g. urchin. From top to bottom are the gland-containing epithelial layer facing the environment, the thick connective tissue-muscle layer, and the peritoneal layer bordering the peripharyngeal coelom. Alcian blue.

layers is a third consisting of connective tissue, nerves, and muscle fibers; this will be referred to as the connective tissue-muscle layer.

The physiology of digestion in *S. purpuratus* has recently been investigated by Farmanfarmaian and Phillips (1962). These workers found that the esophagus and first part of the stomach were the chief sites of digestion and absorption of C^{14}-labeled constituents of the alga, *Iridaea*.

Materials and Methods

All the specimens of *Strongylocentrotus purpuratus* used were collected at low tide from tide pools near Yankee Point, California. Specimens not sacrificed fresh from the field were kept in containers of running sea water until used, and fed as much of the brown alga, *Macrocystis*, as they would eat.

Preliminary preparation of the tissues for histochemical and autoradiographic procedures was the same. Pea-sized urchins, averaging 0.3 g. fresh weight, were fixed whole in 50 ml. of sea water-Bouin's fluid. The sea water-Bouin's fluid decalcified the urchins completely in three days. Application of mild suction removed residual gas bubbles from the urchins after decalcification was complete. These specimens were then washed in water, dehydrated in ethanol, cleared in toluene, embedded in paraffin, and serially sectioned. Larger urchins, ranging from 5 to 20 g. (20 to 35 mm. in test diameter), were fixed in 150 ml. of sea water-

Figure 4. Vertical section of a buccal papilla of a 0.3-g. urchin. From top to bottom are the gland-containing epithelial layer bordering the buccal cavity, the connective tissue-muscle layer, and the peritoneal layer bordering the peripharyngeal coelom. Alcian blue.

Figure 5. Cross-section of the pharynx of a 0.3-g. urchin surrounded by all five interradial buccal diverticula. The section is in the plane of B-B′ in Figure 1. The spaces between the diverticula and pharynx are continuous with the peripharyngeal coelom, as can be seen from Figure 1. At this level the pharynx has a massive connective tissue support in each radial region. The pharyngeal lumen contains a bite of algae. Haematoxylin and eosin.

Figure 6. Cross-section of the pharynx of a 4.5-g. urchin. Only one complete interradial sector of tall epithelium and two radial grooves are shown; each radial groove is subdivided into several groovelets. At the left is the lumen of the pharynx, and next to this is the gland-containing epithelial layer. This epithelial layer is tall in the interradial areas and short in the radial grooves. To the right of the lining epithelium is the connectve tissue-muscle layer, which is thick interradially and thin radially. The peritoneal layer borders the peripharyngeal coelom at the right. Alcian blue.

Figure 7. Cross-section of the pharynx of a 4.5-g. urchin in the same orientation as Figure 6. In this Figure, the radial grooves are subdivided into two groovelets and the space between the lining epithelium and the connective tissue-muscle layer is an artifact. A number of coelomocytes may be seen wandering through the wall of the gut (arrows). Azure A.

Figure 8. Autoradiogram prepared from a cross-section of the esophagus of a 4.5-g. urchin killed one hour after injection of $Na_2S^{35}O_4$. The section has been stained through the emulsion with azure A. The lumen of the esophagus is at the top of the picture. The luminal border of the epithelium stains with azure A, showing beta metachromasia. A heavy concentration of silver grains lies over the middle third of the epithelial layer. At the base of the tall epithelium is a thin connective tissue-muscle layer in which several coelomocytes may be seen. Below the connective tissue-muscle layer is the peritoneal layer, which borders the perivisceral coelom.

Figure 9. Autoradiogram prepared from a cross-section of the esophagus of a 4.5-g. urchin killed 18 hours after injection of $Na_2S^{35}O_4$. The section has been stained through the emulsion with azure A. The orientation is the same as Figure 8, except the zone of silver grains lies over the luminal half of the epithelial layer.

Boum's fluid, after 5- to 10-mm. diameter holes were opened on opposite sides of their tests at the ambitus to permit rapid entry of the fixative. After overnight fixation, the specimens were washed in water and dehydrated as far as 70% ethanol. At this point the urchins were dissected and segments of the following gut regions were removed for further processing: pharynx, esophagus, stomach with attached siphon, intestine, and rectum. The dehydration of the dissected tissues was then completed and they were subsequently cleared, embedded, and sectioned. For examination of the buccal cavity of larger urchins, the lantern and adjacent peristome were removed and processed as a unit like the small urchins.

Urchin gut regions, sectioned at 5 microns, were stained as follows:

(1) The periodic acid-Schiff (PAS) method for aqueous solutions was used according to the instructions of Humason (1962, p. 33). To remove any glycogen which might have survived histological processing, sections, prior to staining, were incubated in a 1% aqueous solution of diastase (malt, USP) for half an hour at room temperature (Pearse, 1960, p. 266).

(2) Alcian blue at pH 3 was used according to directions of Humason (1962, p. 269), omitting the counterstain. Some sections were stained sequentially with alcian blue and then PAS.

(3) Azure A (0.01%) was used according to instructions of Casselman (1959, p. 55). Some sections were stained sequentially with PAS and then azure A.

(4) The mercuric bromphenol blue method for aqueous solutions was used according to the directions of Mazia, Brewer and Alfert (1953).

(5) Sections adjacent to those used for histochemistry were stained routinely with haematoxylin and eosin.

For an autoradiographic investigation of the gut mucopolysaccharides, 100 μc. of $Na_2S^{35}O_4$ (specific activity = 142 millicuries per millimole) were obtained from New England Nuclear Corp., Boston. The S^{35}-sulfate came dissolved in slightly over 1 ml. of distilled water. The distilled water was evaporated by drying the solution for several hours in a 90° C. oven. The S^{35}-sulfate was then redissolved in 0.5 ml. of sea water, which closely approximates sea urchin coelomic fluid in composition. The sea water solution of S^{35}-sulfate was injected into the perivisceral coelom by way of the peristomial membrane. Four urchins (each with a fresh weight of 9 g.) each received 1 μc. of S^{35}-sulfate per gram. Two urchins (each with a fresh weight of 4.5 g.) each received 5 μc. of S^{35}-sulfate per gram. Injected urchins were returned to sea water until killed. Of the animals injected with 1 μc. per gram, one was killed in one hour, one was killed in 18 hours, and two were killed in 24 hours. Of the animals injected with 5 μc. per gram, one was killed in one hour and one was killed in 18 hours. These S^{35}-sulfate-treated urchins were then processed histologically. Sections of gut regions cut at 5 microns (as well as 5-micron control sections from urchins not treated with S^{35}-sulfate) were covered with Kodak AR-10 autoradiographic stripping film. Autoradiograms of guts of animals injected with 1 μc. per gram were exposed for 6 months, while autoradiograms of guts of animals injected with 5 μc. per gram were exposed for 47 days. Some autoradiograms were left unstained and mounted in the aqueous mounting medium described by Boyd (1955, p. 214) for phase contrast observation. Other autoradiograms were stained through the emulsion by the azure A procedure mentioned above and mounted in Permount.

Results

Two of the mucopolysaccharides of the gut of *S. purpuratus* are widely distributed and need not be discussed separately for each gut region. The first of these mucopolysaccharides is contained in the spherules of certain coelomocytes wandering through the connective tissue-muscle layer and inner epithelium of the gut. These spherules stain orthochromatically with azure A, do not stain with PAS or alcian blue, and do not incorporate S^{35}-sulfate. A further discussion of these coelomocytes is beyond the scope of this paper. The second of the widely distributed mucopolysaccharides is found in the connective tissue-muscle layer of all gut regions. This layer stains with PAS after diastase digestion. The strength of the PAS reaction varies from urchin to urchin, ranging from weak to intense. The stronger the connective tissue-muscle layer stains with PAS, the stronger it stains with mercuric bromphenol blue. This layer does not stain with azure A or alcian blue and shows no uptake of S^{35}-sulfate in autoradiograms of S^{35}-sulfate-treated urchins. These results match the criteria defining neutral mucopolysaccharides in mammals (Spicer, 1963). The results with mercuric bromophenol blue suggest that this neutral mucopolysaccharide is associated with a protein, although the specificity of mercuric bromphenol blue for proteins has been questioned (Baker, 1958; Kanwar, 1960).

The histochemical tests demonstrate mucopolysaccharides in unicellular mucous glands in the inner epithelium of all gut regions preceding the junction of the esophagus and stomach. These mucous gland cells rarely exceed 6 microns at their point of greatest width, while their heights vary depending on their location and the size of the animal. The tallest mucous gland cells in a 0.3-g. urchin are 45 microns tall, while the tallest mucous gland cells in a 20-g. urchin are about 150 microns tall. These measurements demonstrate that the thickness of the gut wall relative to the weight of the animal is greater in small urchins than in large urchins. Some types of gland cells have mucopolysaccharides only in their luminal portions, and their unstained basal portions, while difficult to see in sections, presumably reach the base of the epithelial layer. The nucleus, when it can be seen, lies just basal to the secretion-swollen portion. The secretions of the mucous gland cells stain only weakly with mercuric bromphenol blue, and, therefore, contain comparatively little protein. The other properties of the mucous gland cells will be described for each gut region and are summarized in Table I.

The peristomial lip. The peristomial lip (or mouth rim), although not a part of the gut proper, is closely associated with it. The peristomial lip is characterized by a tall, glandular epithelium which distinguishes it from the rest of the peristomial membrane (Figs. 1 and 3). Each gland cell of the peristomial lip is as tall as the epithelium is thick. The basal half of each cell is swollen with a secretion, which reaches the exterior through the narrow neck of the cell. All peristomial lip mucous glands stain with PAS and with alcian blue. They do not stain with azure A and do not incorporate S^{35}-sulfate. The histochemical properties of this secretion do not correspond exactly to the properties of any known mammalian gut mucopolysaccharide (Spicer, 1963). However, it is probable that the peristomial lip gland cells contain a nonsulfated acid mucopolysaccharide containing some PAS-reactive residues or a mixture of neutral mucopolysaccharide and nonsulfated acid mucopolysaccharide components.

TABLE I

Summary giving the location in the gut and the nature of the secretion(s) for each type of mucous gland cell

Gut region	Location within gut region	Type of mucous gland cell	Nature of secretion(s) of mucous gland cell
Peristomial lip	Throughout	Type 1	Nonsulfated acid mucopolysaccharide (and neutral mucopolysaccharide?)
Buccal cavity	Buccal papillae	Type 1	Nonsulfated acid mucopolysaccharide (and neutral mucopolysaccharide?)
	Radial buccal epithelium	Type 1	Strongly acidic sulfated mucopolysaccharide
		Type 2	Sulfated acid mucopolysaccharide
Radial buccal diverticula	Throughout	Type 1	Nonsulfated acid mucopolysaccharide (and neutral mucopolysaccharide?)
		Type 2	Sulfated acid mucopolysaccharide (and neutral mucopolysaccharide?)
Interradial buccal diverticula	Throughout	Type 1	Nonsulfated acid mucopolysaccharide (and neutral mucopolysaccharide?)
		Type 2	Sulfated acid mucopolysaccharide
Pharynx	Radial grooves	Type 1	Strongly acidic sulfated mucopolysaccharide
	Interradial sectors	Type 1	Nonsulfated acid mucopolysaccharide (and neutral mucopolysaccharide?)
		Type 2	Sulfated acid mucopolysaccharide (and neutral mucopolysaccharide?)
Esophagus	Throughout	Type 1	Strongly to moderately acidic sulfated mucopolysaccharide

The buccal cavity. Just within the mouth opening, the wall of the buccal cavity is thrown into numerous protuberances which we shall call buccal papillae. The gland cells in the epithelium covering each buccal papilla are not so abundant as the glands of the peristomial lip, and the secretion-containing part of the gland may occur at any level in the epithelial layer (Fig. 4). The secretion of the buccal papillae glands has the same properties and presumably the same composition as the secretion of the peristomial lip glands already described. In each interradial region just aboral to the buccal papillae, the buccal cavity lining thins to a squamous epithelium at the point where the tooth pierces it. In each radial region aboral to the buccal papillae, a tall epithelium lines the buccal cavity on either side of the opening to the radial buccal diverticulum. Here the buccal epithelium contains two kinds of mucous gland cells. The first type of gland cell predominates, crowding the epithelial layer from base to lumen. These cells show gamma metachromasia

when stained with azure A, but do not stain with PAS or alcian blue. Autoradiograms show extensive uptake of S^{35}-sulfate has occurred in these glands in urchins killed 18 and 24 hours after injection of the radioisotope. These results match the criteria defining strongly acidic sulfated mucopolysaccharides in mammals (Spicer, 1963). The second type of mucous gland cell is less abundant than the first type, and its secretion-containing part is limited to the luminal third of the buccal epithelium. This second type of cell stains orthochromatically with azure A, but does not stain with PAS or alcian blue. Silver grains are located over this second type of gland cell in autoradiograms. Unfortunately, the proximity of this second cell type to the very radioactive first cell type, coupled with the low resolution of S^{35}-autoradiograms, makes it impossible to determine if any S^{35}-sulfate is incorporated by the second type of gland cell. However, in the light of results from other gut regions, these cells probably do incorporate moderate amounts of S^{35}-sulfate. The properties of the secretion of the gland cells of the second type are quite different from any known for mammalian gut mucopolysaccharides (Spicer, 1963). It is probable that the secretion is a sulfated acid mucopolysaccharide.

The radial buccal diverticula. The tall epithelium lining each radial buccal diverticulum contains two kinds of gland cell. The secretion-swollen part of the first kind of gland cell is located in the basal two-thirds of the epithelium and communicates with the lumen by way of a narrow neck. The secretion of these cells has the same properties and presumably the same composition as the secretion of the peristomial lip glands already mentioned. The secretion-filled portion of the less abundant, second type of gland cell is located in the luminal half of the inner epithelium of each radial buccal diverticulum. These cells stain orthochromatically with azure A, but do not stain with alcian blue. Some of these cells give a strong PAS reaction, while others give no PAS reaction. These cells incorporate moderate amounts of S^{35}-sulfate in urchins killed 18 or 24 hours after injection of the radioisotope. The orthochromatic material in these gland cells of the second type is probably a sulfated acid mucopolysaccharide. The PAS-positive material in some, but not all, of these cells could mean that the orthochromatic secretion is heterogeneous, some of it having many PAS-reactive residues and some of it having few PAS-reactive residues. On the other hand, the PAS could be staining a second type of secretion, a neutral mucopolysaccharide coexisting in some cells with the sulfated acid mucopolysaccharide.

The interradial diverticula. The tall inner epithelium of the interradial diverticula contains two types of gland cell. The secretion-swollen portion of the first type of gland cell is located in the basal one-fourth to one-half of the epithelium. Each of these gland cells communicates with the lumen *via* a narrow neck. The secretion of this first type of mucous gland cell has the same properties and presumably the same composition as the secretion of the peristomial lip glands already described. The secretion-swollen portion of the second type of gland cell occurs abundantly in the luminal one-half to three-fourths of the inner epithelium of the interradial buccal diverticula. The secretion of these cells stains orthochromatically with azure A, but does not stain with PAS or alcian blue. Autoradiograms prepared from sections of the interradial diverticula of urchins killed one hour after administration of S^{35}-sulfate show moderate uptake of the radioisotope by the middle third of the epithelial layer. At this level, the epithelial layer contains both

the necks of the mucous gland cells of the first type and the most basal portions of the secretion-swollen parts of mucous gland cells of the second type. In the light of results reported below for the pharynx and esophagus, the radioactivity of the middle third of the epithelial layer is probably due to uptake of S^{35}-sulfate by the basal portions of the secretion-swollen parts of mucous gland cells of the second type. Autoradiograms prepared from sections of radial buccal diverticula of urchins killed 18 and 24 hours after injection of S^{35}-sulfate show a moderate to heavy concentration of the radioisotope throughout the luminal half of the epithelium. Thus, the second type of mucous gland cell in the interradial buccal diverticulum probably contains a sulfated acid mucopolysaccharide.

The pharynx. The pharynx has five longitudinal grooves, one in each radial region. The epithelial cells lining each pharyngeal groove are conspicuously shorter than the tall epithelial cells in each interradial sector of the pharynx. Each radial groove of the pharynx oral to arrow C in Figure 1 is usually single—this condition is shown in Figure 5. Each radial groove of the pharynx aboral to arrow C in Figure 1 is always subdivided into at least two groovelets (Fig. 7) and sometimes more than two groovelets (Fig. 6). The epithelium lining the radial grooves of the pharynx aboral to arrow C in Figure 1 is a continuous sheet of mucous gland cells. These gland cells are so closely packed that boundaries of individual cells are hard to see in sections. The portion of these mucous cells bordering the pharyngeal lumen is filled with a secretion which stains with azure A, showing gamma metachromasia (Fig. 7). This metachromatic secretion never stains with PAS, but sometimes stains weakly with alcian blue. Autoradiograms prepared from sections of pharynx from urchins killed one hour after injection of S^{35}-sulfate show moderate uptake of the radioisotope in the middle third of the radial groove cells. Autoradiograms prepared from sections of pharynx from urchins killed 18 or 24 hours after injection of S^{35}-sulfate show large amounts of the radioisotope localized in the luminal half of the radial groove cells. The properties of the metachromatic secretion of the radial grooves almost match the criteria defining strongly acidic sulfated mucopolysaccharides in mammals (Spicer, 1963). Only the tendency to stain with pH 3 alcian blue indicates that this sulfated mucopolysaccharide of the urchin may be a little less acidic than the mammalian strongly acidic sulfated mucopolysaccharides which Spicer's criteria define.

The tall epithelium of the interradial sectors of the pharynx contains two kinds of mucous gland cell. The first type of mucous gland cell is swollen at the level of the basal third of the tall epithelial layer and empties its secretion by way of a tenuous neck. Just before reaching the lumen, the diameter of the neck enlarges slightly, a feature which may be seen in Figure 6. The secretion of such a cell has the same properties and presumably the same composition as the secretion of the peristomial lip glands already described. The secretion-swollen part of the second type of mucous gland cell occurs in the luminal two-thirds of the tall interradial sectors of the pharynx. This second cell type is abundant only in those parts of the interradial sectors of tall epithelium near the radial grooves, as Figure 7 shows. The secretion of this cell type stains orthochromatically with azure A and stains strongly with PAS; it does not stain with alcian blue. Autoradiograms prepared from sections of pharynx from urchins killed 18 or 24 hours after injection of S^{35}-sulfate demonstrate a moderate amount of the radioisotope in areas of epithelium

which contain this second type of cell. The second type of interradial pharyngeal gland cell is, therefore, comparable to some gland cells of the radial buccal diverticula. That is, each cell may contain a sulfated acid mucopolysaccharide rich in PAS-reactive residues, or it may contain sulfated acid mucopolysaccharide (perhaps similar to the sulfated acid mucopolysaccharide of the interradial buccal diverticula) plus a neutral mucopolysaccharide.

The esophagus. If a segment of living esophagus is turned inside out and viewed with a dissecting microscope, the exposed epithelial layer resembles the surface of a shucked ear of maize. Each area of epithelium resembling a kernel on the ear of maize corresponds to the tall epithelium seen in histological sections of the esophagus. The narrow channels which delimit the "kernels" correspond to the grooves seen in histological sections of the esophagus. Figure 8 shows two areas of tall epithelium separated by an esophageal groove. Since the connective tissue-muscle layer at the base of the tall epithelium is as thin as the connective tissue-muscle layer at the base of the short, groove-lining epithelium, the esophageal grooves are due entirely to differences in the height of the epithelial layer. There is only one kind of esophageal mucous gland cell, which is abundant in the grooves and in the tall epithelium. Azure A always stains the luminal portion of the esophageal mucous gland cells. The width of the azurophilic zone varies from urchin to urchin (Figs. 8 and 9), although, in a given individual, the width of the zone is relatively constant throughout the course of the esophagus. The azurophilic secretion usually shows gamma to beta metachromasia, but in some individuals it may show orthochromasia. In general, the wider the zone that stains with azure A, the greater its tendency to stain orthochromatically. The azurophilic secretion of the esophageal mucous gland cells never stains with PAS, but usually stains weakly with alcian blue. Autoradiograms of sections of the esophagus of urchins killed one hour after injection of S^{35}-sulfate show moderate uptake of the radioisotope by the middle third of the epithelial layer (Fig. 8). Autoradiograms of sections of the esophagus of urchins killed 18 or 24 hours after injection of S^{35}-sulfate show a heavy concentration of the radioisotope in the luminal half of the epithelium (Fig. 9). The properties of the azurophilic secretion in the luminal part of the esophageal gland cells indicate that it is a sulfated acid mucopolysaccharide. The acidity of the secretion appears to vary from urchin to urchin. In some urchins the acid is strong (comparable in strength to the radial groove secretion of the pharynx), but the acid may be of intermediate strength in those urchins which have an esophageal secretion staining orthochromatically with azure A.

The stomach, siphon, intestine and rectum. In none of these urchin gut regions does the inner epithelium contain unicellular gland cells filled with mucopolysaccharides. In some urchins, the luminal border of these gut regions may stain weakly with alcian blue. The source of this apparent acid mucopolysaccharide is unclear. It could be synthesized locally by epithelial cells which are not recognizably differentiated mucous gland cells. On the other hand, this acid mucopolysaccharide could well be produced by mucous gland cells in gut regions preceding the junction of the esophagus and the stomach, and then be carried into the gut regions following this junction to coat the inside wall of the gut.

Discussion

In this paper it has been assumed that S^{35}-sulfate is incorporated only into sulfated mucopolysaccharides and not into proteins. Sulfated mucopolysaccharides and proteins are the only sulfur-containing molecules synthesized by animals and known to survive fixation and subsequent histological processing. The possible incorporation of S^{35}-sulfate into sulfur-containing amino acids, and subsequent use of such amino acids to synthesize proteins is considered very improbable for several reasons. Prosser and Brown (1961, p. 86), after a survey of the amino acid requirements of animals, conclude: "The most striking feature is the similarity in the [amino acid] requirements of all animals. Apparently the general pattern of loss of synthetic capacity of some nine (or ten) amino acids was established very early in animal evolution." Although amino acid requirements are not known for any echinoderm, it is most unlikely that they deviate from the clear-cut pattern seen in other animals. One of the essential amino acids which no animal is known to synthesize is the sulfur-containing amino acid, methionine. Animals can convert methionine into the sulfur-containig amino acids, cysteine and cystine, by a series of irreversible reactions. These three amino acids are the only sulfur-containing amino acids known to be involved in protein synthesis; none of them can be synthesized from sulfate by animals. Moreover, S^{35}-sulfate injected into the sea urchin fails to label the lantern, body wall, axial organ or gonads (which were unripe or immature in the urchins injected). It is especially important to note that the soft, aboral growing tips of the teeth do not label with S^{35}-sulfate. These tooth regions are the sites of intense cell proliferation, and should have a high rate of protein synthesis. If S^{35}-sulfate could be incorporated into proteins by the sea urchin, it should be incorporated wherever proteins are being synthesized, and not exclusively incorporated by regions of gut which contain mucopolysaccharides.

The mucous secretions of the gut of *Strongylocentrotus purpuratus* have the same general features as the gut mucous secretions of *Echinus esculentus* (Stott, 1955) and *Strongylocentrotus intermedius* (Fuji, 1961). In all these regular echinoids, the gut mucous secretions, most of them acidic, are produced by unicellular gland cells of the inner epithelium of gut regions preceding the junction of the esophagus and the stomach; following this junction, there are no mucous gland cells. Stott stained *Echinus* mucous gland cells with a haematoxylin and with mucicarmine. These stains unfortunately lack histochemical specificity. Fuji, however, used several good histochemical tests in his study of *Strongylocentrotus intermedius*. He stained gut mucopolysaccharides with PAS after salivary digestion (which is comparable to diastase digestion), and with toluidine blue (which is comparable to azure A). Fuji, who did not investigate the buccal cavity, claimed that both the esophagus and the radial grooves of the pharynx were crowded with gland cells containing a neutral mucopolysaccharide. In *Strongylocentrotus purpuratus*, the mucous gland cells of the esophagus and those of the radial groove of the pharynx contain no detectable neutral mucopolysaccharide. Therefore, there are interspecific differences in the details of mucous gland cell distribution and content in regular echinoid guts.

Table I shows that the radial buccal epithelium, the radial buccal diverticula, the interradial buccal diverticula and the interradial sectors of the pharynx each

contains two kinds of gland cell. These gland cells were treated as two distinct cell types to facilitate exposition in the results section of this paper. However, it is possible that, in each gut region mentioned, the two mucous gland cell types are actually two successive stages in the secretory cycle of one type of gland cell. Anderson (1960, p. 381) has previously raised the possibility that morphologically different mucous gland cells in one region of an asteroid gut may represent different secretory phases of a single population of gland cells. In the case of the sea urchin gut, we have, at present, no convincing evidence for or against such a secretory cycle.

Sulfated acid mucopolysaccharides of the mammalian gut stain metachromatically with azure A, and nonsulfated acid mucopolysaccharides stain orthochromatically with azure A (Spicer, 1963). Sea urchin gut mucopolysaccharides differ markedly from mammalian gut mucopolysaccharides in their reactions to azure A. Nonsulfated acid mucopolysaccharides of the sea urchin gut do not stain at all with azure A, while sulfated acid mucopolysaccharides of the sea urchin gut may stain either metachromatically or orthochromatically with azure A. In the urchin gut, there is incorporation of S^{35}-sulfate wherever there is azurophilia of any kind. Apparently, in the urchin, a molecule of the orthochromatic sulfated mucopolysaccharide may incorporate detectable amounts of S^{35}-sulfate as sulfate esters without creating enough free electronegative surface charges to cause metachromasia. This might be due to a steric configuration that occludes the sulfate groups.

Mucopolysaccharide biosynthesis probably occurs by a complex series of steps. In the case of sulfated mucopolysaccharides, it is still not known whether the sulfation step occurs before or after polymerization of the sugars. It is known, however, that a compound rich in S^{35} appears in the vesicles of the Golgi complex of mammalian chondrocytes within three minutes of injection of S^{35}-sulfate (Porter, 1964). The S^{35}-sulfated sugars of such a compound would have to be either polymerized or protein-bound in order to survive fixation. The Golgi-associated vesicles might be the actual site of sulfation or they might be a center for rapid concentration or assembly of material sulfated elsewhere in the cell. With the passage of time, the S^{35}-sulfated material is detected in larger vesicles and finally is secreted from these vesicles into the extracellular environment (Porter, 1964). A similar type of secretory activity has been demonstrated for the Golgi apparatus of plant cells (Mollenhauer and Whaley, 1963). Here, secretion vesicles form at the edges of the stacked cisternae. These secretion vesicles pinch off from the cisternae, and become larger and more electron-dense as they move through the cytoplasm to the plasma membrane where their contents are secreted from the cell. The Golgi apparatus is further implicated in secretion by the findings of Hollman (1963), who investigated the ultrastructure of goblet cells, mucous gland cells of the rat intestine. He concluded (p. 547) that "there seems to be no doubt that the Golgi apparatus plays a predominant role in the secretion process of the goblet cell." In at least some of the mucous glands of the sea urchin's gut, the Golgi apparatus may play an important part in the secretion process. Mucous gland cells in several gut regions of the urchin show initial uptake of S^{35}-sulfate in the middle third of the cell (Fig. 8), and a subsequent migration of the label to the luminal part of the cell (Fig. 9). Therefore, either the sulfation step in the synthesis of some sulfated acid mucopolysaccharides or a concentration

of sulfated products occurs in the gland cell region presumably containing the Golgi apparatus.

Mucous gland cells of the esophagus and of the pharyngeal radial grooves are refractory to specific histochemical tests for sulfated acid mucopolysaccharides in the region of initial S^{35}-sulfate uptake. In 18 hours, the sulfated material has migrated to the luminal parts of the cells and stains positively for sulfated acid mucopolysaccharides. A similar phenomenon was described by Immers (1961) for mucopolysaccharides in eggs and embryos of the sea urchin, *Paracentrotus lividus*. Immers claimed that these sulfated acid mucopolysaccharides were masked (*i.e.*, refractory to specific histochemical tests) when combined with protein, and that they became unmasked and stainable when split from the protein; French and Benditt (1953) proposed a molecular mechanism for protein masking of mucopolysaccharides. On the other hand, the region of initial sulfate uptake could be refractory to specific histochemical tests for sulfated acid mucopolysaccharides simply because the sulfated molecules are present in very small amounts, which are detectable autoradiographically, but not histochemically. If we accept the explanation of Immers, we may speculate that, in the sea urchin, sulfated acid mucopolysaccharides of some gland cells may be elaborated (or concentrated) in the Golgi apparatus in combination with a protein. The protein-mucopolysaccharide complex may subsequently migrate (perhaps *via* secretion vesicles) to the luminal part of the cell, where it dissociates, unmasking the sulfated acid mucopolysaccharide.

In *S. purpuratus* the mucous gland cells of the gut have several probable functions. These cells produce mucopolysaccharides which may lubricate the inner wall of the relatively narrow pharynx and esophagus. Such lubrication would protect the delicate gut wall during the passage of hard, sharp objects ingested, such as bites of encrusting coralline algae. Furthermore, the mucopolysaccharides produced in the buccal region of the urchin may well play an important part during ingestion of friable food material. When the teeth bite into a brittle object like a coralline alga, small particles, otherwise lost to the urchin, may stick to mucous secretions surrounding the teeth and thus get drawn into the buccal cavity.

Summary

1. A neutral mucopolysaccharide, probably associated with a protein, occurs in the connective tissue-muscle layer of all gut regions of the purple sea urchin.

2. In the inner epithelium, mucopolysaccharides are found in unicellular glands located in all gut regions preceding the junction of the esophagus and stomach. Such glands never occur in gut regions following this junction.

3. Many, and perhaps all, of the mucopolysaccharides of these unicellular glands are acidic. Of these acid mucopolysaccharides, some are sulfated and others are not.

4. Autoradiograms show that some gland cells which contain acidic sulfated mucopolysaccharides first incorporate S^{35}-sulfate in the middle third of the cell. In some cases, the initially-sulfated material is refractory to specific histochemical tests for sulfated acid mucopolysaccharides, perhaps because the mucopolysaccharides are masked by combination with protein.

5. Autoradiograms show a migration of sulfated material from the middle third to the luminal portion of some gland cells. In cases where the sulfated material was masked when synthesized in the middle third of the cell, it becomes unmasked when it reaches the luminal part of the cell.

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